METHODS FOR STUDYING EARLY LIFE HISTORY STAGES OF ANTARCTIC FISHES

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This methodology report was produced following a meeting at Muséum national d'Histoire naturelle, Paris, France (1-4 September 1981) in collaboration with : J. de Ciechomski, G. Duhamel, G. Hubold, A. Kellermann, C. Moreno, A. North, C. Ozouf-Costaz, M. Rembiszewski, R. Rivoharinala and M. White.

OBJECTIVES FOR INVESTIGATING YOUNG ANTARCTIC FISH

There are six main reasons why ichthyoplankton surveys are required in the Southern Ocean :

1 – Fish are key components within the Antarctic marine ecosystem and knowledge of their early history is fundamental to a complete understanding of their biology.

2 – Ichthyoplankton surveys are an efficient and cost-effective method to demonstrate the species presence in a given area, to estimate the diversity and to evaluate the abundance of selected species.

3 – The surveys should be directed toward a target species (or a group of closely allied species, like Nototheniids of Channichthyids) in order to use the distribution and abundance of eggs or earliest larval stages to obtain an estimate of the biomass of the adult spawning population. This point is particularly important within several areas where active commercial fishing occurs, e.g. South Georgia and Scotia Sea or Kerguelen Islands.

4 – The larvae of a target species should be studied in order to estimate the success of the year class and evaluate the factors influencing variations in survival.

5 – To achieve the preceding points, it is necessary to be able to identify the eggs and larvae of target species. Ichthyoplankton surveys provide suitable material for taxonomic research so that target species can be properly identified. This is important for Nototheniids whose species are closely related. Artificial culture of known species is strongly recommended to confirm identification of early development stages and to estimate the rate of the development.

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6 – Another reason is to use ichthyoplankton in systematic investigations : several recent contributions on fish larvae, other than Antarctic, have shown that the larval characters are among the more useful in showing phylogenetic relationships between genera and families. This is probably the case for Nototheniid fishes.

METHODS OF COLLECTION

Type of gear and recommended techniques

In the Southern Ocean, several types of nets have been used in the recent years : Bongo net, RMT 8 (rectangular midwater trawl), Neuston net, FLH, LHPR (Longhurst/Hardy plankton recorder), HST (High speed townet), pelagic commercial trawl, Hensen closing net. All these nets have their advantages and disadvantages.

The Bongo net is a system which could be used without specialized handling equipment on the vessel acting as a research platform. The RMT 8 when fully instrumented (with flowmeters and opening-closing device) is probably one of the most efficient quantitative nets. The Hensen closing net and the FLH are thought to be more or less interchangeable since they have similar characteristics when used for vertical hauls and have the advantage of maintaining the specimens in good condition. The Neuston net has been noted as a means of collecting some Nototheniid larval stages otherwise difficult to obtain. The HST and LHPR could produce samples of larval material but it is often damaged owing the speed of operation or the manner of accumulating samples. The commercial krill net, a 10 mm mesh trawl, produces large samples of young fish and the later postlarval stages of development.

The following gear and techniques are recommended for ichthyoplankton surveys on the Southern Ocean :

a/ The two recommended nets are the Bongo net and the RMT. Table I indicates the principal characteristics of all the nets already used in the Southern Ocean.

b/ It is strongly recommended that a standard mesh size be used : 0,5 mm is a minimum in the antarctic waters to avoid or reduce problems of phytoplankton clogging.

c/ It is also recommended that closing nets equipped with flowmeters should be used if quantitative estimates of biomass and abundance are required.

d/ Eliminating the bridle preceding the mouth may reduce specimen avoidance. Bongo and RMT nets are suitably designed equipment.

e/ Oblique tows are best to estimate number of eggs and larvae, when estimates of abundance in the entire water column are desired.

f/ Standard rates of wire release and retrieval during net tows are recommended where possible (see Table I).

g/ The depth of tow will depend on sampling location. Over the Antarctic continental shelf, a tow to within 5 metres of the bottom and tows at known depths over the shelf are recommended, while in deep waters sampling should take account of the oceanographical conditions and the target species. Particular reference should be made to the water stratification.

Table I: Characteristics of the nets used in the Southern Ocean

Name	mesh-size mm	type of tow	towing-speed	release rate m/mn	retrieval rate m/mn	Mouth opening m2
Bongo net	0.3 - 0.5	oblique	2	50	20	
RMT	0.5 - 2.0	horizontal or single-descending-oblique	2,5	50	20	8
Hensen	0.3 - 0.5	vertical	–	50	50	0,785
FLH	0.5	vertical or oblique	– 1,5	50 50	50 20	0,785
Neuston net	0.5 - 1.0	horizontal	2-3	–	–	0.045
HST	0.5	horizontal	4-8	50	20	
LHPR	0.5 - 2.0	horizontal	2	50	20	
« krill net »	10	double-oblique				

h/ The towing speed depends on the net used (see Table I) but a balance should be used between a lower speed (risk of increasing avoidance) and the higher speed (risk of damaging the specimens).

i/ A record of the depth and pattern of each tow should be obtained by attaching a Time-Depth Recorded (TDR) to the net at each station.

j/ In some selected areas, e.g. where fisheries are already developped or near a shore station, fixed stations should be established in order to determine gradients of abundance as a function of the time.

k/ Sampling frequency depends on the target species but year-round sampling is strongly recommended because, a priori, the period of spawning is unknown and also because the development of eggs and larvae in the Antarctic waters can be very long (sometimes several months). Year-round sampling is important because the strongly seasonal production cycle results in seasonal growth rates and reproductive cycles.

Use of shore based research facilities

The use of shore based researcn facilities throughout the year or SCUBA techniques in shallow coastal areas could greatly enhance the quality of larval fish research and expand knowledge in the development and distribution od demersal species. Laboratory facilities at shore research stations enable physiological, experimental and developmental research which is not possible from research vessels and so complement the field sampling programmes.

METHODS OF PRESERVATION AT SEA

The following methods are recommended for the antarctic fish larvae:

1 – Preserve specimens as soon after capture as possible to avoid damage and deterioration.

2 – A dye (Rose Bengal) can be used to cut sorting time by 50 % and increase efficiency of sorting. It stains the eggs and larvae a light rose colour and make it easy to pick up out all specimens from a catch. The dyeing is reversible if transferred to clean formalin. The dye can be added to the total formalin mixture for field use.

3 – If possible anaesthetise the specimens before fixation. MS 222 (manufacturer: SANDOZ S.A., Basel, Switzerland), at 1 g for 5 to 10 litres of seawater is suitable.

4 – Fix in 10 % buffered formalin for 14 hours with the specimens kept as flat as possible, as this assists subsequent observations.

5 – Store

a/ in 5 % buffer formalin or

b/ in Strassburger - Flemming solution :

- 100 % alcool	1 part
- Glycerol	1 part
- Distilled water	1 part

c/ or in Newmarch medium:

- Water	70 %
- Glycerol	25 %
- 40 % Formaldehyde	5 % (= commercial formalin)

6 – The second solution (b) appears to be advantageous. Alcohol can be used especially where investigators are allergic to formaldhyde but this preservative leaches out a significant proportion of the colour in small larval stages. So the following media could be used instead they keep the colour of fish and can be used as an alternative:

a - 20 g Potassium nitrate KNO_3
- 10 g Sodium chloride NaCl
- 10 g Sodium sulphate Na_2SO_4
- 10 g Sodium acetate CH_3COONa

dissolved in 1000 ml hot water.

After cooling, add 30 ml formalin and 20 ml glycerol and filter.

b - 0,2 g Ascorbic acid
2 g Ethylenediamine tetraacetic acid (EDTA)
150 mg Butylhydroxyanisol
50 ml Propylene glycol
2 to 4 % Formalin
distilled water: complete to 1000 ml.

7 – Labelling with full information must be done carefully.

METHODS OF TREATMENT IN THE LABORATORY

Usually little additional treatment is necessary except to view by transmitted light. Large specimens can be viewed by reflected light.

Staining with alizarine red S

Specimens for staining should be preserved in a fluid at about pH 9. The method here described is taken from Russell (1976: 29): « After removal from the preservative the specimens should be washed under running tap water for 24 hours. They should then be passed through the alcohols up to 70 %. The stain is made by dissolving and stirring alizarin in about 93 % alcohol until the saturation point is reached ; this would be approximately in the order of 1-2g alizarin in 500 ml alcohol.

« The alizarin solution is added to the 70 % alcohol in which the specimens are lying in the proportion of 5-10 ml stain to 500 ml 70 % alcohol. For best results, the material should stand for about 24 hours in a warm place - up to 25°C. During the process an occasional shake should be given.

« Material can then be taken up through more concentrated alcohol to xylol for clearing. Specimens mounted is neutral canada balsam will keep their stain for years. Raitt recommends the addition of 0,5-1,0 ml of 1 % acetic acid to 500 ml of the staining bath.

« If the specimens are overstained so that the flesh is coloured, this may be washed away with 70 % alcohol, or if excessive with acid alcohol.

« An alternative method, useful for the study of calcified skeletal elements, is that of Hollister. The specimens are first placed in 1 %-4 % potassium hydroxide to clear. 0,1 ml of a stock solution is then added per 100 ml. The stock solution is made up of 96,5 ml water, 13 ml glycerol, 3,5 ml acetic acid and 0,8 ml chloral hydrate, to which alizarin Red is added to saturation. When the bones are stained the specimens are transferred back into the 1 %-4 % potassium hydroxide, and glycerol is then gradually added until the liquid medium is almost pure glycerol.

The melanophore pigmentation disappears, however, during the process, unlike the previously described alcohol method in which the black pigmentation lasts permanently. »

X-rays

This is useful with specimens from about 30 mm total length once the skeleton becomes calcified. Exposure at 3 mA, 40 kV for 18 seconds has produced successful results with the X-ray film Kodak Industrex M or Agfa-Gevaert Structurix D4 DW.

TERMINOLOGY

Considering the difficulties to standardize the terminology used to describe the young stages of fish larvae, several recognized terminologies have been discussed (Moser and Ahlström, 1970 ; Balon, 1975 ; Russell, 1976) and the latter one is recommended for studying the young stages of Antarctic fishes. *Each stage* may have a number of phases. Four stages are recognized :

Embryo : before hatching
Larva : young stage after hatching, with visible yolk-sac
Post-larva : without yolk-sac
Juvenile : individual with full complement of fin rays and majority of characters found in adult.

Figures 1 to 4 show the different stages defined.

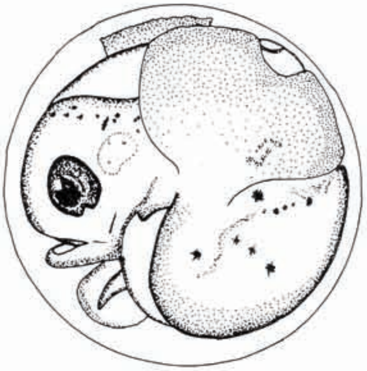

Fig. 1 : Typical embryo of a Nototheniid fish

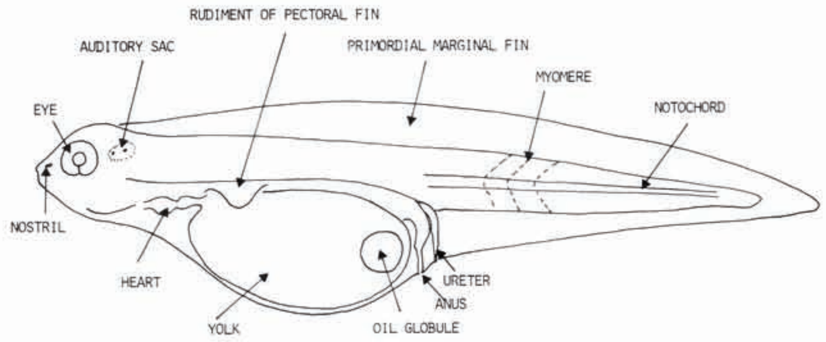

Fig. 2 : Larval stage, with anatomical terminology (from Russell, 1976)

The recommended anatomical terms are those proposed by Miller *et al.*, 1979 (fig. 3-4).

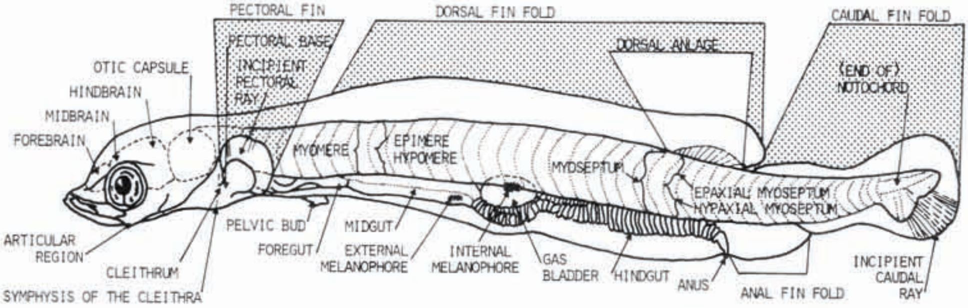

Fig. 3 : Anatomical terminology of a postlarval stage (from Miller *et al.*, 1979)

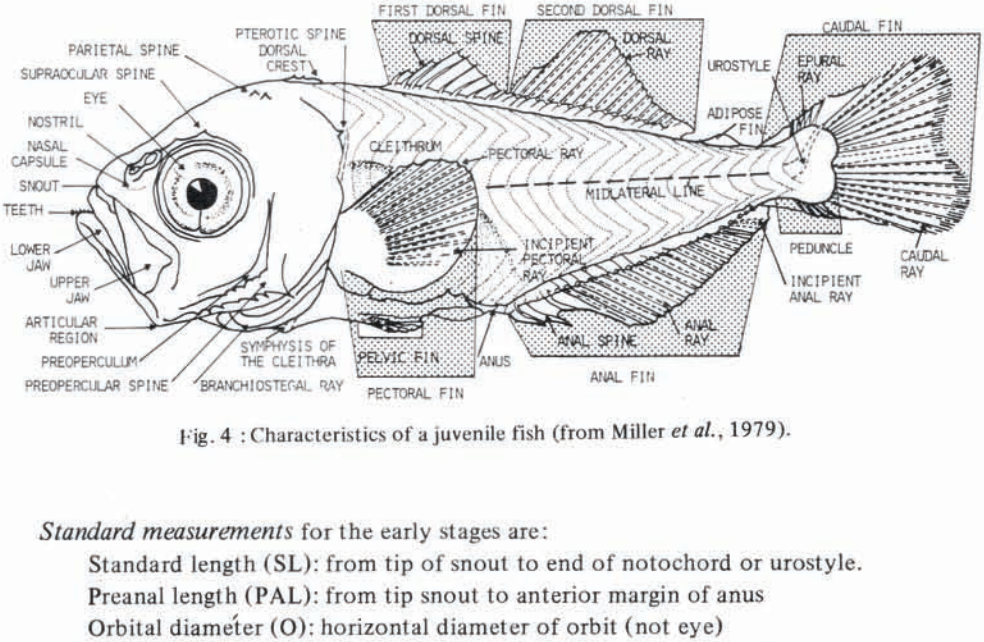

Fig. 4 : Characteristics of a juvenile fish (from Miller *et al.*, 1979).

Standard measurements for the early stages are:

Standard length (SL): from tip of snout to end of notochord or urostyle.
Preanal length (PAL): from tip snout to anterior margin of anus
Orbital diameter (O): horizontal diameter of orbit (not eye)
Preorbital length (POL): from tip of snout to anterior margin of orbit
Head depth (HD): from symphysis of the cleithra to top of head.

The markings on embryos and young stages of fish are due to the presence of pigment cells. The terminology recommended for describing melanophore distribution in Antarctic post-larva is the following one, according to Russell (1976).

Epidermal melanophores

Supra-orbital: on the upper surface of the head above the eye
Ante-orbital, post-orbital: in front of, behind the eye
Snout: on the anterior point of head (upper or lower jaw)
Mandibular: along the lower jaw

Opercular: on the operculum or preoperculum

Contour: Melanophores may occur singly or in rows (single or double) along the *dorsal* and postanal *Ventral* contour of the body.

Lateral: on the sides of the body, melanophores may be situated between the contour row and the centre line of the body. They can be dorsolateral, ventrolateral or mediolateral.

Abdominal: Melanophores may be distributed over the sides or contour of the abdomen preanally.

Throat: In the region just behind the operculum on the ventral surface, melanophores may be present on the isthmus.

Fin: The presence or absence of melanophores on the fins is often a diagnostic character. To describe their position, indicate the fin and whether the melanophores are along the rays or between the rays. On the caudal fin, pigment may be epural (above the urostyle) or hypural (below the urostyle).

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